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IDENTIFICATION AND DRUG SENSITIVITY OF TUBERCLE BACILLI FROM ADDIS ABABA, ETHIOPIA

by

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Summary — *M. africanum* was not encountered among 184 strains of tubercle bacilli isolated in Addis Ababa.

Among strains isolated from treatment failures and relapses the incidence of drug resistance is high : over 40 % to INH, SM and PAS.

KEYWORDS : Tuberculosis; Drug Resistance, Microbial; Mycobacterium; Ethiopia.

Introduction

The frequency of *M. africanum* in several African countries is now well documented (Castets, M., *et al.*, 1963; Castets, M., *et al.*, 1969; Pattyn, S. R., *et al.*, 1970). Since thusfar no studies have been published on Ethiopian strains, we decided to examine a series of 200 consecutive strains isolated in Addis Ababa. At the same time part of these strains were examined for drug sensitivity.

In Addis Ababa sputum cultures for tuberculosis are performed for :

a) all TB suspects whose sputum is negative three times on direct examination in order not to miss any case;
b) patients who fail to improve after treatment (sputum positive) and those relapsing, for the purpose of sensitivity testing.

Consequently most of the strains examined in this study are from patients who had been under treatment for many months. Primary resistance in Ethiopia has not been studied yet. The treatment of tuberculosis in Ethiopia is ambulatory with the following drug regimen. Initial therapy, 3 months : daily streptomycin (15 mg/kg body weight), INH and thiosemicarbazone (1 tablet containing 300 mg of INH and 150 mg of TSC). Sometimes thiosemicarbazone is replaced by PAS 10 g daily. Continuation therapy 9 months : INH + TSC daily or INH + PAS. Injections of SM are supervised, drug taking is not.

Materials and methods

Two hundred consecutive isolates of tubercle bacilli, isolated at the Central Laboratory, Addis Ababa were sent to Antwerp.

Upon arrival the strains were transferred to a tube of Loewenstein-Jensen (L. J.) medium without malachite green, a control tube of L. J. medium and a tube of L. J. medium containing 25 μg thiophen-2-carboxyhydrazide (TCH). After 2 or 3 weeks the cultures were examined for the

type of growth, niacin production and nitrate reduction using techniques previously described (Pattyn and Portaels, 1972).

Approximately half of the strains were tested for drug sensitivity by a simplified method (Kleeberg, in preparation). In this procedure bacilli were transferred onto drug containing L. J. tubes with a 3 mm platinum loop, rubbed over the surface, the latter being « cleaned » of all visible bacteria with the flamed loop. Each drug was tested on one tube, there was one control tube. Drug concentrations were those used in the proportional method (Canetti *et al.*, 1969). After 3 weeks incubation, growth on drug containing tubes was compared with the control, the latter giving confluent growth.

This procedure has been evaluated in a collaborative study on simplified methods for drug sensitivity testing under the auspices of the International Union against Tuberculosis (Coordinator H. H. Kleeberg). Our results were in agreement with the classical method for 97 per cent.

Results

1. *Identification*

Of the 200 strains sent, 184 could be examined, some strains were lost because of breakage during transport, contamination or failure of growth after transfer.

Table 1 shows the results of the identification tests. All strains except one were eugonic and produce niacin, 24 strains were sensitive to 25 μg TCH/ml. Thus there were no *M. africanum* nor *M. bovis* among the strains examined. Thirteen strains failed to reduce nitrate even after repeated testing. These strains were eugonic, and TCH resistant. The occurrence of eugonic, niacin producing, TCH resistant *M. tuberculosis* failing to reduce nitrate has been mentioned previously among *M. tuberculosis* strains from Rwanda (Pattyn *et al.*, 1970) and seems to be peculiar to the highlands of Central Africa.

TABLE 1

Characters of 184 Ethiopian strains of Tubercle bacilli

	Niacin production	Nitrate reduction	TCH resistance
+	183	171	160
–	1	13	24

All strains were eugonic

2. *Drug sensitivity*

In the present study the following drugs were tested : isoniazid (INH) para-amino-salicylic acid (PAS), streptomycin (SM) and thiosemicarbazone (TSC). A small number of strains were also tested for sensitivity to kanamycin (KAN) and rifampicin (RMP). Six strains (3 multiple resistant ones and 3 sensitive ones) were tested in parallel by the proportional technique and the simplified method. Results from both methods were identical.

Fourty six per cent of the strains are resistant against INH, SM or PAS. Resistance to TSC and kanamycin is lower : around 30 per cent. Rifampicin was tested as a control on 66 strains, since this drug has not been used

against tuberculosis in Ethiopia. All 66 strains were found to be sensitive to RMP. Double and triple resistances are as shown in table 2. 20-28 per cent of the strains show a double resistance against INH and either SM, PAS or TSC. The frequency of triple resistance against these drugs is of the order of 15 per cent.

TABLE 2

Double and triple resistance among Ethiopian strains of *M. tuberculosis*

	Double resistance	%	Triple resistance		%
INH + PAS	27/109	25	INH + PAS + TSC	16/109	14
			INH + PAS + KAN	5/66	7
INH + TSC	28/112	20	INH + TSC + SM	18/112	16
			INH + TSC + KAN	6/66	9
INH + SM	32/112	28	INH + SM + PAS	17/109	15

It was possible to separate part of the strains for which the drug sensitivity had been determined into strains originating from relapsing patients and from treatment failures. These results are shown in the second part of table 3. There are no significant differences between the two groups. This is also the case for double of triple resistances (not tabulated). This could mean that most of treatment failures are due to primary resistance.

TABLE 3

Drug sensitivity of Ethiopian *M. tuberculosis*

	INH	SM	PAS	TSC	Kana	RMP
Number resistant/ number examined	52/112	52/112	52/109	33/113	21/66	0/66
% resistance	46	46	47	29	31	0
Relapses	17/42	17/42	20/40	13/42	12/28	0/28
%	40	40	50	31	42	0
Treatment failures	12/23	8/23	8/21	5/23	1/6	0/6
%	52	35	38	21	17	0

Discussion

M. tuberculosis strains in Ethiopia are of the classical type with some strains showing a lack of nitratase. Such strains have also been observed in Rwanda (Pattyn *et al.*, 1970).

Drug resistance is extremely high in the strains examined. However these cultures were derived, mainly from treatment failures and/or relapses. Strains isolated from treatment failures correspond to primary resistant infections, while those isolated from relapses correspond to secondary resistance. Unfortunately among the strains examined it was not possible to distinguish between these two groups.

In Addis Ababa the defaulter rate among tuberculosis patients during treatment is about 50-60 per cent. Thus the figures for the high resistance rates and the high defaulter rate are parallel, illustrating once more that the magnitude of the resistance problem is a measure of the succes of TB control programmes. In the Ethiopian control programme every effort should be concentrated on regularity of treatment since this should prevent emergence of drug resistance.

Identification et résistance médicamenteuse de bacilles tuberculeux isolés à Addis Ababa, Ethiopie.

Résumé — Parmi 184 souches de bacilles tuberculeux isolés à Addis Ababa il n'y eut aucune souche de *M. africanum*.

La résistance médicamenteuse parmi les souches isolées à partir d'échecs de traitement ou de récidive est extrêmement élevée : plus de 40 % vis-à-vis de l'INH, SM ou PAS.

Identifikatie en geneesmiddelenresistentie van tuberkelbacillen afkomstig van Ethiopië. Addis Ababa.

Samenvatting — Onder 184 tuberculose stammen uit Addis Ababa werden geen *M. africanum* gevonden.

De stammen geïsoleerd uit gevallen van mislukte behandelingen en recidieven vertonen een zeer hoge resistentie : 40 % tegenover INH, SM en PAS.

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